Table S1. Biosamples for multi-omics profiling of EIMD.

Sample Type	Description & Advantages	Limitations	Example Applications (References)
Muscle biopsy (tissue)	Invasive percutaneous biopsy of skeletal muscle (e.g. vastus lateralis). Provides direct measurement of local molecular changes (transcripts, proteins, metabolites) within the affected muscle [1]. Yields high tissue-specificity and the ability to assess fiber-type differences and intracellular signaling events in situ.	Invasive and limited by sample size – repeated biopsies can cause additional tissue damage and are not always feasible [2]. Biopsy captures a snapshot in time and may not reflect systemic responses. Requires careful handling to preserve labile metabolites.	- **Transcriptomics/Proteomics:** Identifying gene expression and protein network changes in muscle after training or damage [1]. - **Metabolomics:** Measuring intramuscular metabolites (e.g. lactate, amino acids) post-exercise [3]. - e.g. Deep proteome analysis of muscle biopsies revealed fiber-type specific adaptations to exercise training [4].
Blood (plasma/serum)	Minimally invasive, systemic fluid reflecting whole-body response. Contains muscle-released enzymes (CK, LDH), cytokines, hormones, metabolites, and extracellular vesicles. Easy serial sampling enables time-course studies (pre- vs post-exercise kinetics). Well-established protocols for plasma proteomics and metabolomics with high throughput.	Systemic dilution of muscle-derived signals; some muscle changes may not be detectable in circulation. Contains abundant proteins (e.g. albumin) that can hamper detection of lower-abundance biomarkers. Biological variability from other organs (e.g. liver metabolism, stress hormones) can confound muscle-specific interpretations.	- **Proteomics:** Profiling circulating protein changes (e.g. inflammation, complement cascade) after exercise or injury [5]. - **Metabolomics:** Global metabolic signatures of exercise intensity/fatigue in plasma (e.g. lactate, fatty acids, TCA intermediates) [3]. - **Exerkine discovery:** Identifying novel exercise-induced secreted factors (e.g. interleukin-6, BDNF, clusterin) that rise in blood and mediate tissue crosstalk [5].
Interstitial fluid (muscle)	Local extracellular fluid within muscle, sampled via **microdialysis** probes or suction blister techniques. Directly reflects the muscle microenvironment – including secreted proteins (myokines), peptides, and metabolites diffusing out of muscle cells during exercise. Can capture early signals before they dilute into bloodstream.	Semi-invasive (requires probe insertion) and low-volume yield. Dialysate samples are very dilute; low abundant proteins may require concentration steps. Temporal resolution is limited by probe equilibration (often 30+ min) so rapid changes might be averaged out. Handling challenges to prevent sample loss or contamination.	- **Proteomics:** Mapping the temporal pattern of muscle-secreted proteins post-exercise [4 6]. - **Metabolomics:** Monitoring local metabolites like glucose, lactate, and amino acids in muscle during exercise recovery, offering insight into tissue-level metabolism independent of hepatic clearance [3].
Urine	Non-invasive waste fluid reflecting systemic metabolic changes. Some muscle breakdown products and metabolites (e.g. creatinine, amino acid catabolites) are excreted in urine, which can serve as integrated indicators of exercise stress and recovery. Easy to collect in large volumes and suitable for	Indirect measure – contains downstream metabolites after renal processing, not real-time muscle milieu. Dilute and subject to hydration status variability. Proteins are present at very low concentrations (most large proteins are not filtered), limiting proteomic analyses	- **Metabolomics:** Several studies have mapped urinary metabolite changes after strenuous exercise or muscle-damaging protocols. E.g. high-intensity rowing induced EIMD and led to 49 significant metabolite changes in urine (23 up, 26 down) related to energy and antioxidant pathways. Some urinary metabolites (alanine, lactate, etc.) show potential as biomarkers for EIMD occurring ~24 h post-exercise [7]. - **Biomarker panels:** Recent work

	metabolomics with minimal sample prep.	(requires concentrating, and only low MW peptides pass). Timing of appearance can be delayed relative to muscle events.	identified multi-metabolite signatures in urine that predicted muscle damage severity with good sensitivity/specificity [7].
Sweat	Non-invasive fluid excreted by sweat glands during exercise. Contains electrolytes, small metabolites (e.g. lactate, urea, ammonia), and trace amounts of proteins/peptides [8]. Sweat collection (e.g. via patches) allows continuous sampling during exercise. Potential for real-time monitoring using wearable sensors (for certain analytes like lactate, glucose).	Very low protein content – sweat proteomics is challenging without enrichment, as many proteins are near or below detection limits. Composition can be influenced by hydration, skin contamination, and sweat rate. Not all biomarkers present in blood will appear in sweat or at quantifiable levels. Data from sweat often have higher noise and variability.	- **Metabolomics:** Profiling metabolites in sweat to distinguish aerobic vs anaerobic exertion (e.g. higher lactate and ammonia in sweat during intense exercise). One pilot study performed combined sweat metabolite and protein analysis, finding correlations in metabolite abundances between individuals [8]. - **Proteomics:** Limited but emerging – small studies identified some sweat proteins that change with exercise (e.g. dermcidin, immune-related proteins), though extensive fractionation was needed. Sweat proteome analysis is currently considered feasible mainly for abundant peptides or with specialized microfluidic collection concentrating sweat.
Other biofluids	Saliva: contains stress hormones (cortisol), IgA, and some metabolites – convenient but few omics studies beyond targeted hormone assays. Synovial fluid: in context of exercise and joint impact (less relevant to muscle damage). Breath condensate: can reflect volatile metabolites (like acetone) from exercise fat oxidation. These niche samples are occasionally explored for specific questions.	Saliva proteins can originate from oral tissues, confounding muscle signals. Low-volume or specialized collection needed for breath and synovial fluid. Analytical sensitivity is a limiting factor for these mediums in multi-omics.	- **Saliva cortisol/genomics:** Used in overtraining studies to assess stress hormone and possibly cell-free DNA changes; but omics in saliva for muscle damage is rare (most saliva studies are in endurance context for dehydration or stress markers). - **Exhaled breath:** targeted analyses show increased ammonia and acetone with intense exercise (reflecting amino acid catabolism and ketosis), measurable by spectroscopy or PTR-MS. Integration into multi-omics is still nascent.

Table 3. Selected multi-omics studies of EIMD/adaptation.

Study (Year) & Cohort	**Omics Layers & Techniques**	**Key Findings** (muscle damage & recovery insights)	**References**
Jang et al. (2018) – 11 young adults (5♂,6♀) performing eccentric exercise (step-down protocol, inducing DOMS)	– Urine metabolomics: ^{1}H-NMR and LC-QTOF-MS at 0h, 2h, 24h, 48h, 72h, 96h post-exercise. – Data analysis: PCA, OPLS-DA for gender/time differences; univariate stats for each metabolite.	– Clear sex-specific metabolomic responses to muscle damage: males showed significant post-exercise increases in alanine, lactate, glycine, creatine phosphate, etc., peaking ~24–48h; females showed a unique increase in adenine only. – Trajectory of metabolite changes aligned with soreness timeline (metabolites spiked as DOMS peaked, then normalized by 96h). – Interpretation: Elevated alanine and lactate indicate heightened glycolysis and amino acid catabolism from muscle breakdown; increased adenine in females suggested nucleotide turnover differences. Proposed alanine, lactate as candidate urinary markers of muscle damage in males.	[7,9]
Deane et al. (2023) – 16 adults (8 young, 8 older) undergoing 20-week resistance training (RET)	– Muscle proteomics: Isobaric Tag (iTRAQ) labeling + nano-LC-Orbitrap MS of vastus lateralis biopsies pre- vs post-training. Also phosphoproteomics via TiO_2 enrichment and MS. – Data analysis: Network analysis for hub proteins; comparison of young vs old responses.	– Identified 73 proteins significantly altered by age and/or training. Older adults had blunted hypertrophy and showed impaired upregulation of cytoskeletal and extracellular matrix proteins after training. In contrast, older muscle did upregulate many metabolic enzymes (glycolysis, mitochondria), partially "rejuvenating" those pathways. – Phosphoproteomics: older adults had baseline differences in muscle signaling (e.g. lower phosphorylation of anabolic signaling proteins); training increased phosphorylation of some targets (AMPK, etc.) but not to youthful levels. – Hub analysis: PYK (pyruvate kinase) was a hub in improved metabolism network, while YWHAZ (14-3-3ζ) was a hub in the network of proteins that remained dysregulated (cytoskeletal assembly) [10]. – Interpretation: Muscle of older individuals preferentially adapts by boosting energy metabolism but fails to fully repair or strengthen structural integrity, potentially explaining higher injury risk. Suggests combining metabolic and structural biomarkers to assess training efficacy in elderly (e.g. citrate synthase activity up = good, but persistent high collagen fragments = incomplete recovery).	[1]
Zhao et al. (2024) – Mice, downhill running + whey protein intervention	– Muscle metabolomics: UPLC-QTOF-MS untargeted profiling of gastrocnemius muscle in mice after exercise, comparing those given whey protein hydrolysate (WPH) vs regular whey vs control.	– Exercise without supplementation led to depletion of TCA cycle intermediates, lipids, and carbs in muscle during 1h recovery. Mice given WPH showed significantly higher levels of amino acids in	[3]

	– Molecular assays: Western blots for mTOR pathway activation (Sestrin2/Akt/mTOR/S6K).	muscle within 1h post-exercise than controls, along with more rapid replenishment of muscle glycogen metabolites and vitamins. – WPH also uniquely elevated certain lipid metabolites linked to membrane repair in early recovery. – Western blot confirmed WPH activated mTOR signaling compared to non-supplemented, indicating faster anabolic signaling onset. – Interpretation: targeted nutrient can modulate the muscle metabolome to favor recovery – providing excess amino acids and energy substrates to damaged muscle, which correlates with immediate activation of protein synthesis pathways. Suggests specific metabolic biomarkers could be used to gauge if muscle is in a pro-anabolic state vs a depleted catabolic state after exercise.	
MoTrPAC (2024) – Rats (both sexes), endurance training 0,1,2,4,8 weeks	– Multi-tissue multi-omics: Transcriptomics (RNA-seq), proteomics (DIA-MS), metabolomics & lipidomics (LC-MS), phospho-proteomics, acetyl-proteomics, and epigenomics (ATAC-seq, DNA methylation) on 18 tissues (muscle, heart, liver, plasma, etc.). ~9,400 assays in total – one of largest exercise omics datasets. – Integration: Comparative analysis across tissues and time; identification of shared vs tissue-specific responses; pathway enrichment across omes.	– Uncovered thousands of molecular changes with training. Many were tissue-specific: e.g. skeletal muscle upregulated oxidative metabolism genes & proteins and showed epigenetic activation at those loci, aligning with improved endurance capacity. Adipose tissue had distinct changes in lipid metabolism transcripts; liver showed alterations in PPAR signaling, etc., reflecting systemic adaptation. – Common pathways: immune modulation and stress-response pathways were widely regulated across multiple tissues. Recovery/injury pathways spiked early in training in muscle and connective tissues, indicating micro-damage and repair, but subsided by 8 weeks as tissues became conditioned. – Data were linked to human relevance: many exercise-regulated genes overlapped with those implicated in diseases. For example, genes improved by training in rats were ones usually suppressed in chronic inflammatory diseases. – Resources: the consortium provided a public database for researchers to query any gene/metabolite's response to exercise. This is accelerating hypothesis generation – e.g. identification of a novel protein (Ccdc136) that was upregulated in muscle and liver; its knockout is now being tested for effects on exercise capacity. – Interpretation: A holistic atlas that confirms exercise simultaneously engages multiple biological networks. It underscores why multi-omics is needed: focusing only on muscle transcriptomics,	[11]

		for instance, would miss parallel changes in plasma metabolites or liver enzymes that are crucial for full adaptation. The integrated data highlight candidate circulating biomarkers that reflect multi-tissue improvements.	
Wu et al. (2025) – 18 untrained young men, high-intensity rowing to induce EIMD	– Urine metabolomics: Quasi-targeted LC-MS (QqQ) for 100+ known metabolites, pre- and immediately post-exercise; follow-up samples at 24h. – Plasma measures: CK, LDH, HBDH (β-Hydroxybutyrate dehydrogenase), plus pain VAS scores to confirm EIMD. – Stats: Volcano plots, pathway enrichment, ROC analysis for biomarker selection. Built predictive models (logistic regression) for EIMD using metabolites.	– Demonstrated that intense rowing caused significant muscle damage (CK rose ~28%, LDH ~10%, VAS soreness appeared at 24h). Urine metabolomics revealed 49 significantly altered metabolites post-exercise (23 up, 26 down). These spanned pathways: energy metabolism (e.g. ↑ketone bodies, ↓TCA intermediates), performance metabolites (↑allantoin, a purine oxidative product), and antioxidant metabolites (↑urate, etc.). – Using an algorithm, they identified a panel of 5 urinary metabolites that best discriminated EIMD vs no EIMD. Notably, 1-stearoylglycerol (a lipid), dihydroorotate (pyrimidine metabolism), D-lactate, allantoin, and naphthoquinone were selected. A multi-metabolite model had ROC-AUC 0.97, outperforming any single metabolite. – Pathway analysis showed enrichment of purine metabolism (consistent with ATP breakdown in muscle), branched-chain amino acid catabolism, and lipid mobilization pathways in those with EIMD. – Proposed stearoylglycerol and D-lactate as potential early urinary markers. These could indicate cell membrane breakdown and altered gut/microbiome activity). – Interpretation: Non-invasive urine metabolomics can predict and reflect muscle damage. Integrating multiple metabolites captures different aspects (energy depletion, cell damage, oxidative stress) giving a robust composite biomarker. This addresses a key controversy – how to predict EIMD early without blood tests. Wu's results are an initial proof that a metabolite cocktail in urine could serve this role, but they note need for validation in athletes and with varying exercise types. Their work also highlights that machine learning is crucial to sift through many metabolites to find the best predictors, underscoring the chemometric theme of multi-omics studies.	[7]

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